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Artemia in aquatic toxicology : a review

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Abstract

Due to the commercial availability of dried cysts from which live test material can be hatched at will, *Artemia* is used extensively in research and applied toxicology.

Despite the extensive literature on dose-effect relationships of chemicals on brine shrimp, it was not until 1980 that an experimental protocol was developed for a simple acute toxicity test with *Artemia* nauplii, meeting the prerequisites for standardization.

The reliability and accuracy of this short-term test were determined during an intercalibration exercise involving 80 laboratories and were found to be quite satisfactory. Consequently, the so-called ARC test, which is one of the very few standardized marine toxicity tests, is now used routinely at the international level.

Recent research on the use of *Artemia* in ecotoxicology has focused on the development of testing procedures and screening bioassays with sublethal responses. The medical, drug, and food sectors seem to use *Artemia* assays as frequently as laboratories investigating environmental concerns.

Toxicity tests with brine shrimp have a significant potential in QSAR research because of their simplicity, rapidity, and cost-effectiveness. *Artemia* tests also have a good predictive potential as alternatives for other crustacean test species.

This review postulates the future role of *Artemia* tests in aquatic toxicology to be that of a reference or quality control in rapid screening tests, as much as that of a predictor of chemical effects on species in marine environments.

Introduction

Artemia continues to be used extensively in research and applied toxicology laboratories worldwide. Uses include the investigation of sources of toxicity in chemical mixtures and environmental samples, the acute screening of chemicals, the detection of natural toxins in foodstuffs and in pharmaceuticals, the study of models of toxic action of substances, and the study of the trophic transfer of pollutants. *Artemia* is proving to be a versatile and valuable organism in single-species toxicity tests, particularly if studied with other endemic species. This brief review describes recent studies, programs, and developments within this wide range of applications and discusses *Artemia*'s future role in basic and applied aquatic toxicology.

Hazard assessment

The hazard resulting from the release of anthropogenic chemicals into aquatic environments is a function of the probability and intensity of the exposure of biological systems to the chemicals, and of the potential of chemicals to harm biological systems, which in turn depends upon the chemical's physico-chemical properties and the unique characteristics of the exposed biota.

Hence, hazard assessment strategies always include two components :

1) the exposure analysis to determine the concentration of the pollutant at a particular time and place ;
2) the effects analysis to determine the negative effects which the chemical may exert on biota living at the site of concern.

Such strategies have been described in many recent documents (*e.g.* Bergman *et al.*, 1986).

Butler (1978) defined ecotoxicology as "the science concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems, including the transfer pathways of those agents and their interactions with the environment". Consequently, testing of the effects of man-made chemicals should in principle always be carried out on multispecies systems, such as micro-ecosystems (*i.e.* microcosms, mesocosms) which simulate natural conditions (National Research Council, 1981 ; Cairns, 1985). Calamari *et al.* (1985), however, portrayed the inverse relationship existing between ecological realism and simplicity of testing in test systems of increasing complexity (Fig. 1). With regard to species and response criteria, Persoone (1980) on the other hand, showed the inverse relationship existing between the ecological realism and the sensitivity and costs of bioassays (Fig. 2). Most of the ecotoxicological knowledge to-date is based on single-species testing, the majority being acute tests for reasons of practicality, reliability, and general application. Bioassays with *Artemia* rank highly as candidates for rapid and cost-effective routine bioassays in hazard assessment schemes incorporating single-species and multiple-species approaches (Hammons, 1981 ; National Research Council, 1981 ; Cairns, 1985).

Development of a short-term *Artemia* test

During the past 30 years, many papers have been published on the effects of chemicals on brine shrimp, using different procedures, response criteria, life stages, and durations of the tests (see updated bibliography on *Artemia* by McCourt and Lavens, 1985). Research on *Artemia* ecotoxicology was initiated in 1975 at the State University of Ghent in Belgium, to evaluate the usefulness and reliability of different published toxicity testing methods with brine shrimp (Vanhaecke *et al.*, 1980). This evaluation and our own experimentation showed that none of the published methods were acceptable for use in a standardized, acute routine test.

Hence, a list of theoretical prerequisites and important parameters was derived for developing a simple and reliable screening test with *Artemia.* Following existing methods, an experimental protocol for a routine toxicity test was developed, called the Artemia Reference Center (ARC) test. Four decisions were made : the type of test was static, the duration was 24 h, the life stages were nauplii, and the response criterion was mortality, expressed as an LC50. After 2 years of research, the accuracy, reliability and reproducibility of the ARC-test were considered to be acceptable. The test was submitted for criticism to a special workshop on *Artemia* toxicity tests

during the First International Symposium on The Brine Shrimp, held at Corpus Christi, Texas, in 1979 (Persoone *et al.*, 1980). The test procedure was considered logical and well-developed. A recommendation was formulated that the reliability, accuracy, and precision of the bioassay in the various laboratories should be determined by a Round Robin (Intercalibration) Exercise (Persoone and d'Agostino, 1980).

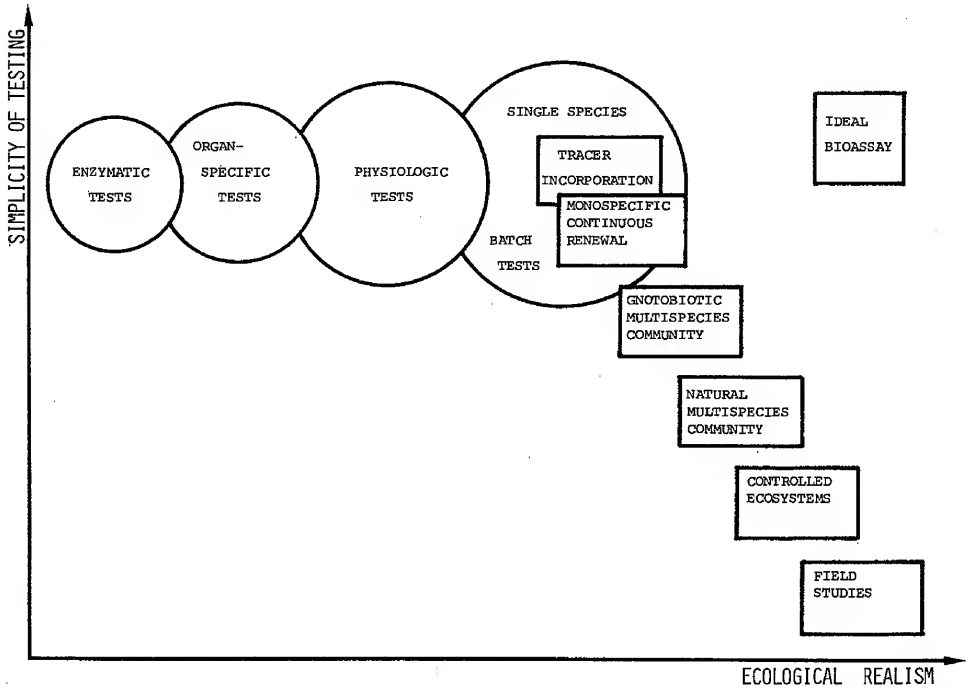


FIG. 1. Inverse relationship between simplicity and ecological realism in test systems of increasing complexity (modified from Calamari *et al.*, 1985).

Intercalibration exercise-ARC test

A call for participation in the Round Robin Exercise was sent to a large number of institutes, laboratories, and companies throughout Europe in late 1980. A similar exercise in North America was launched from the Freshwater Institute, Winnipeg, Canada. Positive replies were received – approximately 100 from Europe and 125 from Canada and the USA. Each laboratory was then provided with materials (cysts, seawater salts, reference chemicals, instructions, reply forms). Sixty European and 20 North American laboratories participated ; the very low response from the American contingency was due to a long postal strike in Canada.

Two points regarding this exercise are important. With 80 replies, this Round Robin on an aquatic toxicity test was the largest study conducted to-date. In addition, for two-thirds of the participating laboratories, the intercalibration exercise was their first experience with *Artemia* as a toxicity test-species. Hence, their personnel had few or no prior skills in hatching cysts, handling nauplii, or making observations during the assays.

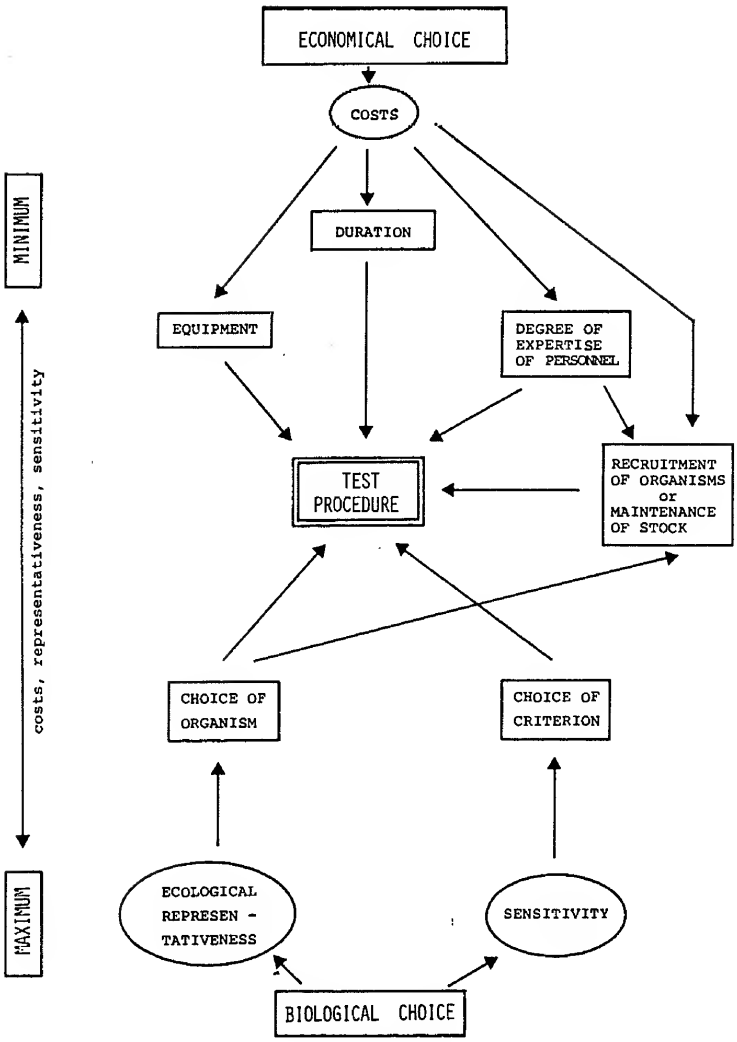


FIG. 2. Interrelationships of the basic factors determining the choice of bioassay test methods (from Persoone, 1980).

Results of the Round Robin were published in a EEC Report (Persoone *et al.*, 1981) and were presented in 1981 at the INSERM Symposium on Acute Aquatic Ecotoxicological Tests in France (Vanhaecke and Persoone, 1981). Most laboratories conducted the prescribed test with relatively few difficulties. Both the intra- and interlaboratory variabilities of the ARC test were satisfactory in comparison to those of other Round Robin tests conducted in Europe for the EEC (*e.g.* the acute *Daphnia* and *Brachydanio* tests, now adopted by the OECD and subsequently endorsed by the EEC). As stated above, two-thirds of the participating laboratories had their first encounter with *Artemia* in this Round Robin, compared to other exercises with *Daphnia* and zebrafish (*Brachydanio rerio*) with which most participants were already familiar. It is likely that with more practice and skill, the repeatability and reproducibility of the ARC test will improve.

The intercalibration exercise was very helpful in identifying weak points in the experimental protocol, resulting in continually improved versions (*e.g.* Wells *et al.*, 1982, 1985). Our collective efforts have led to an acute screening-testing protocol of intermediate sensitivity, satisfactory repeatability and reproducibility, low cost, minimum maintenance of animals, and universal, year-round applicability.

Martox – standardization of marine toxicity tests

In 1983, an International Symposium on "Ecotoxicological Testing for the Marine Environment" was convened at the State University of Ghent to determine the state of the art of marine ecotoxicology (Persoone *et al.*, 1984). One discussion session at the Symposium was devoted to standardization. It was apparent that, with the exception of the acute *Artemia* nauplii test, Woelke's oyster test (Woelke, 1972), Reish's polychaete test (Reish, 1984), and echinoid assays (Kobayashi, pers. commun.) very few marine tests could be considered as standardized. Relatively few Round Robin tests have been carried out thus far with marine species, the number of laboratories participating is small, and the results are often disappointing. With other zooplankton, this situation is now changing, notably for tests with larvae of mysids, copepods, decapods, and echinoids. This is particularly due to the involvement and interest in the United States, of ASTM, APHA, and EPA in standardizing acute toxicity tests. Hence, there soon should be a data base with which to compare *Artemia versus* other species on key aspects of standard protocols.

Considering the advantages of using *Artemia* as a test species for routine bioassays, one would expect wide use, especially for regulatory purposes. In fact, regulatory use at present is largely limited to the 1978 EEC Directive on the dumping of titanium wastes, which prescribes – without giving any experimental protocol – that next to "tests for acute toxicity on certain species of molluscs, crustaceans, fish, and plankton" bioassays should be carried out with larval and adult brine shrimp. In addition, the EPA continues to use *Artemia* for testing oil spill dispersants, along with other crustaceans and fish. Some international conventions, such as the Oslo Convention, have recently excluded *Artemia* as a test organism from their sets of mandatory or recommended bioassays. Consequently the organism and the testing protocol have had a mixed reception.

There are several reasons for opposition to using *Artemia* in regulatory hazard assessments. *Artemia* is not present in the sea, thus it is not a natural or endemic marine organism. However, *Artemia* is highly euryhaline ; it can be cultured at salinities of 5 up to 150 ‰. Since it is not competitive with other zooplankton, it is mainly found in high salinity biotopes, not those of typical estuaries and coastal waters. A second reason is that *Artemia*, because of its specialized tolerance to high salinities, is presumed not to be very sensitive to contaminants. This is usually correct for the mortality criterion, especially compared to other microcrustaceans such as *Pseudocalanus minutus* (see next section). It is debatable however, whether this reason negates the many advantages that *Artemia* offers as a test organism in acute screening assays. This is particularly true when, for some toxicants, the sensitivities of other species are predictable from the *Artemia* data (Wells *et al.*, 1982 ; Abernethy *et al.*, 1986). The third reason for opposition is that some experimenters have had little success with *Artemia*, probably due to incorrect techniques for hatching the cysts and manipulating the nauplii during holding and in experiments ; this reason is particularly invalid for rejecting a valuable reference test organism.

We are convinced that, if the standard ARC test was better understood, improved upon by individual investigators, and used actively as one of several marine, single-species screening tests, it would find gradual acceptance as a reference test in the array of toxicity tests and approaches needed for national and international pollution research and control. Interestingly enough, *Artemia* seems to be included more often in manuals describing bioassay procedures for testing chemicals and effluents. The recent US-EPA methods document for testing acute toxicity of industrial effluents (Peltier and Weber, 1985) includes *Artemia* for both food and test organisms. In Canada, Environment Canada (EPS) lists *Artemia* as one of its suggested zooplankton toxicity tests (MacGregor and Wells, 1984). The ARC test is slowly but surely being adopted and used in more laboratories for research, screening, and regulatory purposes.

Developments in ecotoxicological research with brine shrimp since the first *Artemia* symposium, 1979

Table I, which summarizes published work in *Artemia* ecotoxicology since 1979, shows that efforts have been considerable including development of testing procedures, screening bioassays, and lethal and sublethal research assays. The last category represents extensive efforts covering many sublethal responses and environmental samples or suspected toxins and toxicants. The medical, drug- and food sectors use the assays as frequently as those laboratories investigating environmental problems. The brine shrimp is used primarily with the classical aquatic toxicology approach, rather than through newer, innovative, multi-species, ecological toxicology. However, the number of reported studies, from many countries, underlines the animals usefulness rather than its limitations.

One area of research for which *Artemia* tests seem to have a significant potential is QSAR (quantitative-structure-activity-relationships). QSAR's have been used extensively and are still used in pharmacology and food science to determine relationships between the structure of related chemicals and their metabolic and toxicological activity within living organisms. The QSAR approach, in use for several decades, has recently been rediscovered and applied by environmental chemists and toxicologists to determine the relationship between selected physico-chemical properties of xenobiotic compounds and their acute lethal and sublethal toxicity (Veith and Konasewitch, 1975 ; Goldberg, 1983 ; Kaiser, 1984). Since QSAR's are in fact based on large series of identically conducted bioassays with many chemicals, in homologous and non-homologous series, it is clear that *Artemia* larvae constitute ideal aquatic test organisms for such research, not the least for cost-effective reasons.

Foster and Tullis (1984) selected the octanol-water partition coefficient as a representative parameter of molecular structure. This factor is used frequently as a rapid predictor of the bioconcentration potential of organic pollutants in water. It also has wide applicability as a predictor of acute toxicity. The acute toxicity to *Artemia* larvae of 11 organic compounds (naphthalene and its derivatives, phenanthrene, pyridine, 1, 2-dichloroethane, chloroform) was determined. A highly positive linear relationship between log P (*i.e.* the octanol-water partition coefficient) and "activity" (log 1/IC50, where IC50 was the median immobilization concentration) was found. The equation ($\log 1/IC50 = 1.57 + 0.88 \times \log P$) was derived. A general equation for the relationship between *Artemia* naupliar toxicity and the partitioning coefficient of chemicals ($\log 1/TR + a + b \times \log P$) was developed, in which TR is the measured toxic response.

TABLE I

Developments in ecotoxicological research with brine shrimp since the first International *Artemia* Symposium (1979)

Category	Reference	Comments
1. Reviews	Grozdov *et al.* (1983)	Description of bioassays used to assess marine pollution. Includes *Artemia*.
	Vanhaecke *et al.* (1981)	Description of methodology of short-term standardized test with nauplii.
	Vanhaecke and Persoone (1984)	
	Wells (1984a)	Brief review of current use and continued development of *Artemia* toxicity procedures.
2. Culture for toxicology	Beck and Bengtson (1982)	Evaluation of five strains of *Artemia* used as diet for Atlantic silversides, *Menidia menidia*, used in toxicological studies. Standard strain is recommended.
	Groat *et al.* (1980)	Culturing of *Artemia* for toxicological studies with *Aurelia aurita* larvae.
	Sleet and Brendel (1983)	Improvement of methods for harvesting and counting nauplii from synchronous populations.
	Leonhard (1981)	Culturing technique for *Artemia* used in toxicology.
3. Development of bioassays	Amiard-Triquet *et al.* (1981)	Development of acute toxicity procedures with *Artemia*.
	Bengtson *et al.* (1984)	Demonstration of *Artemia* diet quality effects on the results of toxicity tests with three species of marine organisms.
	Denuit *et al.* (1982)	Study of the effect of developmental stage on *Artemia* sensitivity to metals.
	Kerster and Schaeffer (1983)	Development of teratogen test system based on disrupted elongation of nauplii, exposed to wide range of contaminants.
	Vanhaecke *et al.* (1980)	Description of seven factors crucial to acceptable reproducibility of a routine, acute toxicity test with nauplii.
	Vanhaecke *et al.* (1981)	Proposal of a standard procedure for acute toxicity test with nauplii.
	Vanhaecke and Persoone (1984)	Detailed description of a standard acute toxicity test with nauplii and evaluation of intra- and interlaboratory variation of results with two chemicals.
4. Screening assays	Adema and Vink (1981)	Comparison of toxicity of dieldrin to three crustaceans. *Artemia* nauplii most sensitive.
	Amiard-Triquet (1983)	Comparison of sensitivities of several developmental stages of several organisms.
	Aubert *et al.* (1983)	Toxicity of silicon compounds to *Artemia*.
	Betz and Blogoslawski (1982)	Evaluation of toxicity of dinoflagellates using an LD50 (ingestion) shrimp test.
	Bijl *et al.* (1981)	Evaluation of mycotoxin toxicity. *Artemia* preferred for simplicity of test to five other species.
	Bijl *et al.* (1982)	Detection of trichothecenes in food with the aid of *Artemia* bioassay.

TABLE I. Continued

Category	Reference	Comments
	Chattopadhyay (1983)	Study of pharmacological activity in isoquinoline-derived alkaloids.
	Cooper *et al.* (1981)	Comparative toxicology of jet fuels to *Artemia* and *Daphnia magna*.
	Eng-Wilmot and Martin (1979)	Toxicity of algal and dinoflagellate cultures to *Artemia*.
	Eng-Wilmot and Martin (1981)	Interactions between algal and dinoflagellate cultures to mitigate toxic effects on *Artemia*.
	Meyer *et al.* (1982)	Use of *Artemia* in simple bioassay of active (*i.e.* toxic) plant constituents.
	Podojil *et al.* (1979)	Use of *Artemia* bioassay to examine human, bacterial and fungal toxins.
	Prior (1979)	Bioassay of mycotoxins in animal feedstuffs with *Artemia* larvae.
	Smolka and Schulz (1980)	Use of *Artemia* bioassay to test isolates of filamentous fungi from apples.
5. Screening assays (QSAR)	Abernethy *et al.* (1986)	Comparative and QSAR-related toxicology of hydrocarbons to *Artemia* nauplii and *Daphnia magna*.
	Foster and Tullis (1984)	Establishment of QSAR relationship between partition coefficients and acute toxicity of naphthalenes and other hydrocarbons, using *Artemia* nauplii.
	Foster and Tullis (1985)	Examination of QSAR relationships in osmotically stressed *Artemia* nauplii exposed to various organic chemicals.
6. Lethal assays (research)	Castritsi-Catharios *et al.* (1980)	Study of effects of several surfactants and one dispersant on hatching and survival of *Artemia*.
	Castritsi-Catharios *et al.* (1982)	Study of toxicity of three surfactants and one dispersant to nauplii-survival and hatching.
	Castritsi-Catharios *et al.* (1984)	Study of toxicity of an oil dispersant on the intestinal epithelium of two strains of *Artemia*.
	Castritsi-Catharios *et al.* (1986)	Comparison of sensitivities of two *Artemia* populations to a dispersant and its mixture with oil.
	El-Zayat *et al.* (1985)	Screening of "biologically active" organic compounds.
	Gaeta *et al.* (1983)	Toxicity of pesticide-mercury mixtures to *Artemia* larvae.
	Jacob *et al.* (1980)	Comparative toxicity of metals, oils, dispersants, mixtures to various species, including *Artemia*.
	Jones (1980)	Acute toxicity tests to nauplii of two drilling mud additives, and comparison to other regulatory toxicity testing protocols.
	Nikonenko and Aivazova (1983)	Toxicity of phenol to several aquatic organisms, including *Artemia*.
	Olney *et al.* (1980)	Analysis of nauplii of *Artemia* from Brazil, Australia, Italy, and USA for chlorinated hydrocarbons. All levels less than 100 ppb on wet weight basis.

TABLE I. Continued

Category	Reference	Comments
	Pankhurst *et al.* (1980)	Fluoride (NaF) inhibited growth of *Artemia* (12 d, 5 ppm), in comparative study with bivalves, krill and sole.
	Persoone *et al.* (1986)	Report on combined effects of temperature and salinity on sensitivity of nauplii to potassium dichromate and sodium lauryl sulphate.
	Suarez *et al.* (1981)	Toxicity screening of fungal strains from starches with nauplii.
	Tanaka *et al.* (1982)	Toxicity study of 17 metallic compounds and their mixtures with mycotoxins.
	Verriopoulos and Moratiou-Apostolopoulou (1983)	Comparison of toxicities of a crude oil, an oil dispersant and its mixture using *Artemia*.
	Weber and Rosenberg (1980)	Examination of toxicity of toxaphene from estuarine sediments to *Artemia*.
	Wells (1984b)	Presentation of acute toxicity data on *Artemia* nauplii and marine copepods exposed to oil spill dispersants.
	Wells *et al.* (1982)	Acute toxicity studies with Corexit 9527 dispersant and mineral oil, on *Artemia* nauplii.
	Wells *et al.* (1985)	Acute toxicity studies with solvent and surfactant components of oil spill dispersants, on *Artemia* nauplii and *Daphnia magna*.
7. Biochemical and physiological assays (research)	Alayse-Danet *et al.* (1979, 1980)	Measurement of variations in enzymes (amylase, trypsin), and growth in *Artemia* exposed to copper and zinc. Enzyme responses were generally more sensitive.
	Austerberry *et al.* (1979)	Study of di-N butyl phthalate hydrolysing enzymes in developing nauplii.
	Castritsi-Catharios *et al.* (1984)	Acute toxicity of four surfactants and an oil spill dispersant.
	Dechev and Matveeva (1978)	Proposal of respiration response as a method for examining toxicity of oils and dispersants.
	Hudson *et al.* (1981)	Study of uptake, metabolism and toxicity of di-N-butyl phthalate to synchronously developing larvae. Extraction of enzymes that may detoxify the phthalate.
	Hudson *et al.* (1982)	Isolation and purification of the hydrolysing enzyme from phthalate exposed larvae.
	Matveeva (1979)	Measurement of respiratory rates of *Artemia* under crude oil and oil products exposures, followed by recoveries in clean water.
	Samain *et al.* (1981)	Measurement of correlations between amylase and trypsin content of *Artemia* (San Francisco strain) and copper toxicity.
	Sleet and Brendel (1982)	Measurement of selective toxicity of model toxicants with different developmental stages.
8. Reproductive and developmental assays (research)	Browne (1980)	Measurement of survival and lifetime reproductive performance in shrimp (five strains) exposed to copper sulphate.

TABLE I. Continued

Category	Reference	Comments
	Kerster and Schaeffer (1983)	Development of teratogen testing system based on disruption of elongation of nauplii, and assay of a wide range of contaminants. Not a very sensitive test.
	Kissa *et al.* (1984)	Estimation of LC50's, and EC50's (hatching rate) of four metals (Cd, Cr, Ni, Co).
	Kuwabara *et al.* (1980)	Development and assessment of hatchability as a test method with approximately 40 contaminants.
	Landau and Rao (1980)	Measurement of effects of precocene II on hatching, survival and activity of nauplii.
	Leonhard and Lawrence (1980)	Application of acute and chronic tests in study of effects of cadmium on reproduction.
	Okasako and Siegel (1980)	Toxicity of sodium chloride, sulphur group (VIa) compounds on hatching of cysts.
	Sleet and Brendel (1983)	Examination of nauplii for potential in teratogen screening tests. Instars I to IV were suitable for indicating developmental effects of inorganics.
9. Food chain assays (research)	Cosson (1979)	Comparison of water versus food routes of contamination by copper, with shrimp, mussels and several fish.
	Komatsu *et al.* (1978)	Food chain experiments with radiation, including phytoplankton, *Artemia*, and several fish.
	Komatsu *et al.* (1981)	Study of accumulation through food chain with diatoms, *Artemia* and Killifish.
	Milner (1982)	Use of *Artemia* in study of zinc accumulation by flatfish.
	Snarski and Olson (1982)	Use of *Artemia* in study of influence of diet on mercury toxicity and bioaccumulation in fathead minnows.
	Wrench *et al.* (1979)	Use of *Artemia* in study of arsenic metabolism in algal-crustacean food chain.
10. Model ecosystem (research)	Higuchi *et al.* (1980)	Assessment of bioaccumulation kinetics and sublethal (growth, fecundity) radiation effects in brine shrimp reared in model ecosystem and exposed to tritium.

Very recently, Mackay and co-workers at the University of Toronto (Abernethy *et al.*, 1986) have used an improved ARC test and the acute *Daphnia* test for QSAR determinations with 37 hydrocarbons and chlorinated hydrocarbons. Good correlations were found between the aqueous solubility of the chemicals and their acute toxicity to *Artemia* and *Daphnia* as expressed by the 24 h LC50 (Fig. 3).

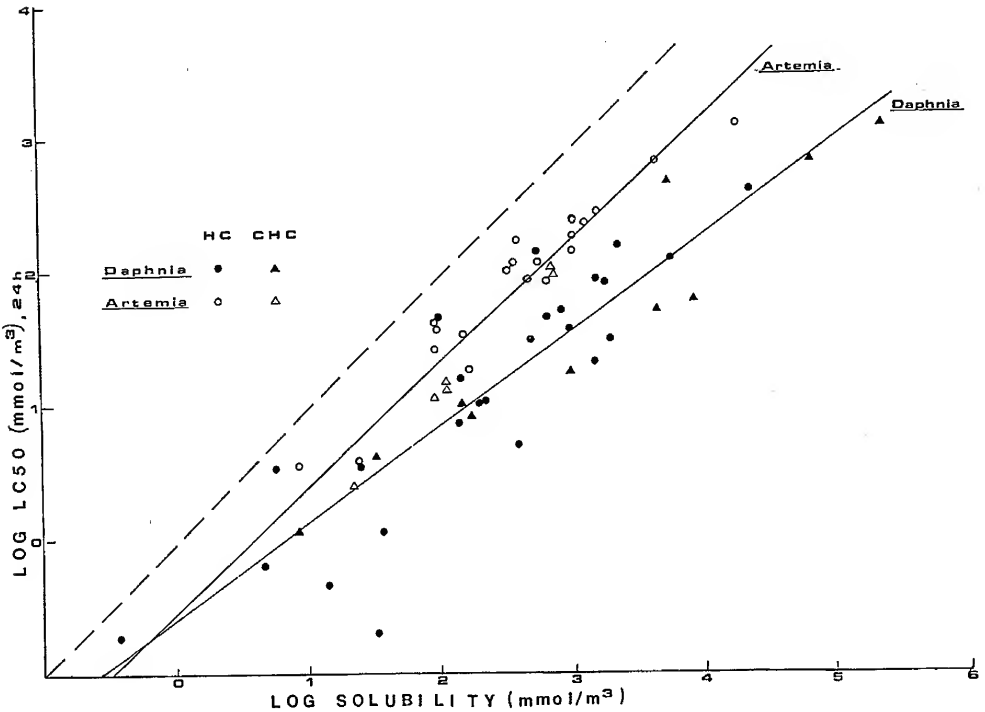


FIG. 3. Correlation between aqueous solubility of hydrocarbons (HC) and chlorinated hydrocarbons (CHC), and their acute toxicity to *Daphnia* and *Artemia* (from Abernethy *et al.*, 1986).

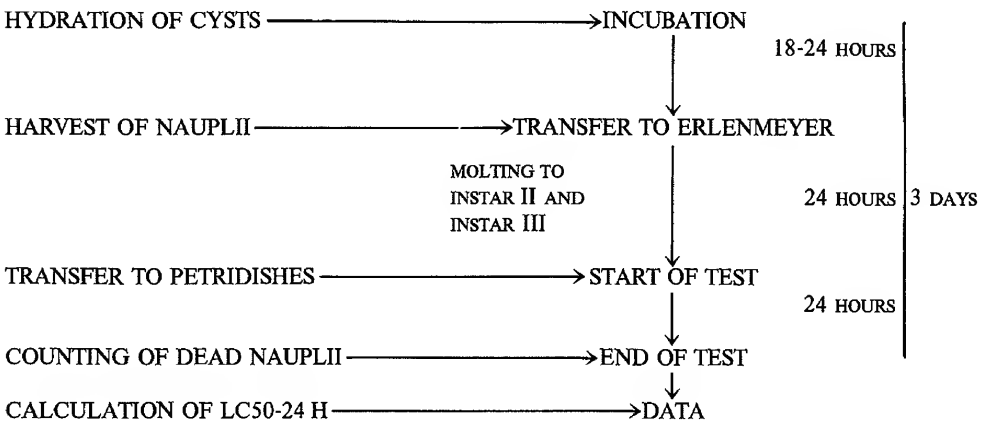
An important conclusion from both studies with *Artemia* and *Daphnia* is that acute toxicities of many organic compounds to crustaceans are largely non-selective. In other words, acute toxicity is not influenced primarily by molecular structure. It is rather correlated with the rate and success of organism-water partitioning of the chemical (Abernethy *et al.*, 1986), which for nonpolar, organic compounds is reflected by aqueous solubility and/or octanol-water partition coefficients. *Artemia's* role in this fundamental research in the QSAR field is underlined here.

Mackay and co-workers in Toronto, Canada, recently also emphasized the predictive potential of *Artemia* tests. During extensive studies with zooplankton including *Artemia*, and oils, oil dispersants and their components, it was discovered that the acute lethal toxicity of a chemical or formulation to *Daphnia magna* and marine copepods was often predictable from the *Artemia* data (Wells *et al.*, 1982, 1985 ; Abernethy *et al.*, 1986).

The advantages of the acute *Artemia* ARC test for routine experimentation in aquatic toxicology have been demonstrated recently at the State University of Ghent (Persoone *et al.*, 1986). Comparative series of acute bioassays (24 h LC50's) have been conducted with three well-known test species – *Daphnia magna, Artemia,* and the brackish water rotifer *Brachionus plicatilis* – to determine the effect which different combinations of environmental variables (temperature, salinity) have on acute toxicities of two chemicals. The *Artemia* part of the comparative study consisted of 150, complete 24 h bioassays, each with eight concentrations, triplicated with 10 nauplii each. With the standard ARC test (Table II), each assay is set up in half an hour and mortalities are counted a day later in half an hour. Both the *Artemia* and the *Brachionus* assays, which could each time be started from inert cysts and were thus independent of continuous maintenance and availability of healthy stock-cultures, were completed before the *Daphnia* tests. This comparative study clearly demonstrated the usefulness of the *Artemia* test for rapidly studying the interactive effects of variables on the toxicities of contaminants.

TABLE II

Schedule for preparation and execution of the ARC-test



The future of *Artemia* ecotoxicology

We have presented the status of the ARC test, current toxicological research with *Artemia,* and promising avenues of ecotoxicological research being explored with *Artemia* in Belgium and Canada. The role of *Artemia* in ecotoxicology, particularly aquatic, is shown in Table III, where the distinction is made between the various applications of the ARC test (*e.g.* screening, comparing, investigating effects of other variables) and the research areas with both standard and unique, continually developing methods (QSAR, teratogenic assays, investigations into modes of toxic action, comparative toxicology, etc.). Although we may have given the impression that *Artemia* is or should be a "key" species in aquatic toxicology, we would like to emphasize that *Artemia*'s usefulness in the hazard assessment of chemicals and environmental samples should be evaluated objectively. No single organism or testing protocol fulfills all criteria to determine the toxicity of materials, and as underlined by Cairns in many papers, there are inherent dangers

TABLE III

Fields of immediate application of the standard ARC-test

- Routine monitoring of ambient waters (freshwater and marine)
- Testing of effluent toxicity prior to release
- Testing of waste toxicity prior to ocean dumping
- Testing of the toxicity of mixtures of chemicals
- Testing of oil and oil dispersant toxicity
- First toxicity ranking of new chemicals and formulations

Research in *Artemia* toxicology

- Determination of QSAR's with various categories of chemicals
- Comparative toxicity studies with other test-species for predictive purposes
- Development of sensitive sublethal bioassay methods (growth, reproduction, physiological and biochemical criteria)
- Development of multispecies tests to study the effects and the dynamics of pollutants between trophic levels
- Development of bioaccumulation tests
- Study of the influences of abiotic and biotic factors on toxicity levels for various categories of chemicals

in single-species approaches, regardless of the species used. *Artemia* has been useful in the past to both research workers and regulators. Perhaps its role is one of being a reference or quality control organism in assays, as much as a predictor of chemical effects on species in marine environments. *Artemia* deserves its place in the battery of test species for aquatic toxicology, and should be used wherever possible to identify, understand or assess, solve, and prevent problems from xenobiotic chemicals. We are confident that in the years to come, more people worldwide will gradually discover the numerous advantages and potential applications of *Artemia* tests in aquatic toxicology.

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